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New claims 9 and 10 are added as follows:

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9. (New) The method according to claim 1, further comprising the step of:
calculating the ratio of the total count of leukocytic cells and erythroid cells to the
count of erythroid cells or leukocytic cells.

10. (New) The method according to claim 1, further comprising the step of:
calculating the ratio of the obtained leukocytic cell count to the obtained erythroid
cell count.

REMARKS

Claims 1-8 are pending in the present application. New claims 9 and 10 have been added
by the present amendment.

Claims 1-3 have been amended and remains supported by the original claims 1-3 and the
specification at, for examples page 4, line 24 to page 5, line 1, page 18, lines 23-25, page 19,
lines 24-27, page 20, lines 11-16, Fig. 1 and Fig. 2.

New claims 9 and 10 have been added, and recite limitations in the original claim 1.

No issue of new matter should arise and entry of the amendment is respectfully requested.

Claims Objections

Claims 1-8 were objected because of the following informalities as the Examiner stated:

Step 1 of claim 1 would be more clear if the two substeps, lysing and staining, had
subletters a) and b);

Step 3 of claim 1 is confusing because it has awkward syntax. There should be a comma
after "erythroid cells".

In response, amendments have been made as the Examiner suggested. Hence, these
objections have been obviated.

Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1-8 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner stated that the calculating steps in steps 4 and 5 of claim 1, steps 7 of claim 2, and claim 3 lacked clear antecedent basis. In the presently amended claims and newly added claims, all the relevant calculating steps are specifically based on the corresponding previous steps of classifying cells and obtaining count thereof. Hence, all the calculating steps now have clear antecedent steps. Withdrawal of these indefiniteness rejections are respectfully requested.

Claim Rejections under 35 U.S.C. § 103

Claims 1-8 are rejected under 35 U.S.C. 103 as being unpatentable over Inami et al. (U.S. 5,298,426) in view of Kim et al. (U.S. 5,559,037), Hansen et al. (U.S. 4,284,412) and Hoffman et al. (U.S. 4,492,752) and further in view of Kim et al. (U.S. 5,516,695). Applicants respectfully traverse this rejection.

Inami et al. teaches a two step method comprising the staining of white blood cells and nucleated red blood cells in a bone marrow fluid by specific nuclear stains. Specifically, this method relies on a two-step staining process that uses a first fluid that is an acidic hypotensive fluorescent dye solution and a second fluid that is a solution that changes the osmolarity and pH of the first fluid. The first fluid further contains a fluorescent dye that diffuses into the erythroblasts of the nucleated red blood cells to specifically stain the erythroblast nuclei, and the erythroblasts are detected with a flow cytometer.

Bone marrow fluid normally contains lipid particles. Inami does not recognize the problem of the presence of lipid particles contained in bone marrow fluid, which reduces the accuracy of counting the nucleated bone marrow cells. In Inami, there is no teaching or suggestion to differentiate the lipid particles from other components of the bone marrow fluid. For example, in the scattergram reported in the examples of Inami, lipid particles are not seen at all. These lipid particles have not been separately classified from nucleated red blood cells.

All the other references including Kim (US 5,559,037), Hansen (US 4,284,412), Hoffman (US 4,492,752), Kim (US 5,516,695) relate to the analysis of the different nucleated cell types in

blood samples by using scattered light and fluorescence. None of these references teaches or suggests to improve the accuracy of analyzing leukocytic and erythroid cells in a sample of bone marrow fluid. Nor is there any teaching or suggestion to detect, classify or quantify the lipid particles that are present among the leukocytic and erythroid cells of the bone marrow fluid in any of these references.

Although blood contains erythroblasts, and the erythroblasts are found primarily in bone marrow fluid as disclosed in Inami, a blood sample is still very different in composition from the bone marrow fluid. Hence, a person of ordinary skill in the art would not be motivated to combine the above-cited references (related to analyzing blood samples) or make any modification based on the teaching in those references, to arrive at the present invention which relates to the analysis of bone marrow fluid. More importantly, even if a person of ordinary skill in the art combines the teachings of these references, he still cannot make the present invention that improves the accuracy of analysis of nucleated bone marrow fluid by separately classifying the lipid particles, leukocyte cells and erythroid cells, as set forth in the presently amended claim 1. Hence, the present invention is not obvious over any of the references cited by the Examiner either considered independently or in combination. In fact, to arrive at the present invention with improved accuracy of analysis, the inventors of the present invention have made an intensive study of the target components in bone marrow fluid that are to be classified and counted, other components competing or interfering with the target components and how they compete or interfere with the analysis of the target components.

Accordingly, the present invention as claimed in the presently amended claim 1 which recites the step of classifying the lipid particles and the leukocytic cells and erythroid cells in the bone marrow fluid is not obvious over Inami and the remaining references cited by the Examiner. Further, at least for the same reason, claims 2-11, depending from claim 1, are also not obvious over Inami and the remaining references cited by the Examiner. Hence, withdrawal of these obviousness rejections is respectfully requested.

Therefore, Applicants believe the present claims, are in a condition of allowance, and respectfully request an early and favorable consideration and allowance of the presently pending claims 1-10.

It is believed that no fees or charges are required at this time in connection with the present application; however, if any fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,
COHEN, PONTANI, LIEBERMAN & PAVANE

By Kent H. Cheng
Kent H. Cheng
Reg. No. 33,849
551 Fifth Avenue, Suite 1210
New York, New York 10176
(212) 687-2770

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AMENDMENTS TO THE CLAIMS SHOWING CHANGES

IN THE CLAIMS:

1. (Amended) A method of classifying and counting [nucleated bone marrow cells] leukocytic cells and erythroid cells in a bone marrow fluid comprising leukocytic cells and erythroid cells and lipid particles, comprising the steps of:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering [and render] leukocytic cells [and], erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence [between] among the leukocytic cells, [and] the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect at least one kind of scattered light and at least one kind of fluorescence;

(3) classifying [and counting] the lipid particles, [nucleated bone marrow cells,] the leukocytic cells and the erythroid cells [with use of the a difference] by the difference in the [intensity] intensities of [the] their fluorescence and [a difference in intensity of the] their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells classified in the step of (3). [calculating the ratio of the nucleated bone marrow cells to the erythroid cells or leukocytic cells from the obtained erythroid cell count or leukocytic cell count and the obtained nucleated bone marrow cell count; and

(5) calculating the ratio of the leukocytic cells to the erythroid cells from the erythroid cell count and the leukocytic cell count.]

AMENDMENTS TO THE CLAIMS SHOWING CHANGES (Cont'd.)

2. (Amended) The method according to claim 1, further comprising the steps of:

[(6)] classifying erythroid cells into at least two erythroid cell groups according to maturity of each of the erythroid cells, and obtaining a count of [counting] cells in each of the erythroid cell groups [with use of] by the difference in the [intensity] intensities of the fluorescence and the scattered light from the at least two erythroid cell groups; and

[(7)] calculating the ratio of the classified cells in each of the erythroid cell groups to [all] the total erythroid cell count [erythroid cells from the obtained erythroid cell count in each of the groups and the total erythroid cell count].

3. (Twice amended) The method according to claim 1, further comprising the steps of:

classifying lymphocytes and monocytes in the leukocytic cells and obtaining a lymphocyte count and a monocyte count; and

[(8)] calculating a myeloid cell count by deducting [a] the obtained lymphocyte count and [a] the obtained monocyte count from the leukocytic cell count; and

[(9)] calculating the ratio of the erythroid cells to myeloid cells from the obtained myeloid cell count and erythroid cell count.